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# Aflatoxins in stored maize and rice grains in Liaoning Province, China

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**Abstract**

Aflatoxin contamination and its relationship to storage length in stored maize and rice in Liaoning Province, northeastern China, was investigated. Aflatoxins in 110 samples collected from an area of 14.68 million $km^2$ covering storage length from 1 yr to over 10 yr were determined by high-performance liquid chromatography with fluorescence detection. The results showed that almost all samples collected contained aflatoxins. The average contents in maize, whole grain rice and brown rice were found to be 0.99, 3.87 and 0.88 $\mu g\,kg^{-1}$, respectively. Three-fourths of the total aflatoxins in whole grain rice (3.87 $\mu g\,kg^{-1}$) could be removed by dehusking to as low as 0.88 $\mu g\,kg^{-1}$ in brown rice. No significant aflatoxin increase was observed in whole grain rice and brown rice over a 10-yr storage period. In maize, the amount of aflatoxins was significantly higher in 2-yr than 1-yr sample. Aflatoxin $G_1$ was detected as the major type of aflatoxin in over 40% of all stored grain samples tested and over 92% of rice samples examined. The aflatoxin content in maize and rice is much lower than the regulated maximum amount allowed in foodstuffs in China and other countries. We concluded that these grains are safe for human and livestock consumption and for trading.


*Keywords:* Aflatoxins; Maize; Rice; Storage length; HPLC

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## 1. Introduction

Aflatoxins are secondary metabolites produced by *Aspergillus flavus* Link and *A. parasiticus* Speare (Bennett and Goldblatt, 1973; Cleveland and Bhatnagar, 1992; Cotty, 1997; Yu et al., 2004a). These fungi survive in a wide range of environments and can be found in soil, in plant and animal remains, and in grains and seeds such as maize, peanuts, and tree nuts (Pitt, 2000). These two fungi are responsible for spoilage of stored grains around the world (Paster, 1995). *Aspergillus flavus* is the main fungus that causes pre-harvest aflatoxin contamination in field crops. The Food and Agriculture Organization of the United Nations (FAO) estimated that at least 25% of the world's cereal grains are contaminated by mycotoxins, including aflatoxins (Dowling, 1997). During the years spanning 1977–1994, high aflatoxin levels (more than 20 $\mu g\,kg^{-1}$) in maize were reported (Moreno and Kang, 1999). Colonization of maize crops prior to harvest in the field (pre-harvest) by aflatoxigenic fungi often resulted in spoilage and aflatoxin accumulation in post-harvest grains during storage (Resnik et al., 1996; Nesci et al., 2003).

Because of the toxic and potent carcinogenic properties of aflatoxins, many developed countries have established very stringent regulations limiting the maximum allowable amount of aflatoxins in food and feed (Table 1, Xiao, 1988; Hansen, 1993). Some developing countries like China and Mexico have also set up regulations compatible with those in the United States for human consumption and for trading (Table 1). Because of the food safety regulations, grains with higher level of aflatoxins are prohibited from trade domestically or internationally (Ellis et al., 1991). Economic losses are significant to farmers. Health issues related to aflatoxin contamination of foodstuffs are more problematic in developing countries where no proper food safety regulation has been established. For both food safety and economic reasons, aflatoxin contamination is therefore a serious concern throughout the world.

An investigation on aflatoxin contamination of agricultural commodities was carried out in different parts of China in 1981 (Liu et al., 1981). Research on controlling aflatoxin contamination in stored rice was also conducted (Ilago and Juliano, 1982). Aflatoxin $B_1$ was detected in stored rice and 96% of parboiled rice (Breckenridge et al., 1986). In southern China, in Fujian and Guangxi Province, a comprehensive survey on aflatoxins in grains (maize and rice) was performed by Tang et al. (1998) and Tang (1999). Aflatoxin contamination generally occurs in the tropical and sub-tropical regions of the world but aflatoxin accumulation in food and feedstuff in northern China (Shandong and Henlongjiang Provinces) was reported (Li, 1997; Zhang et al., 2003). Some feedstuffs and maize from Liaoning Province were found to contain aflatoxins (Dai, 1997). Maize and rice are the main diet of the general population in Liaoning and most parts of China. To date, there have been no comprehensive surveys on aflatoxin content in Liaoning Province. In this investigation, the current situation of aflatoxin contamination in stored

Table 1
Maximum amount of aflatoxins allowed in foodstuffs in some countries (unit, $\mu g\,kg^{-1}$) for human consumption and for trading

| Australia | China | EU | France | Germany | Holland | India | Japan | Malaysia | Mexico | UK | US |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 2 | 10 | 5 | 5 | 30 | 10 | 35 | 20 | 10 | 20 |

grains in Liaoning Province, one of the three major grain production Provinces in northeastern China, has been examined.

## 2. Materials and methods

### 2.1. Grain samples

A total of 110 grain samples of maize and whole grain rice was collected at 65 sites from bulk grain grown and stored locally in Liaoning Province from mid-August to early October in 2003. To avoid sampling error due to the highly heterogeneous nature of fungal distribution, each 6 kg composite sample collected from one storehouse was a mixture of 15 sub-samples (400 g each). The 15 sub-samples were collected from five diagonal points on each of the upper, middle and lower layers of each storehouse. There were 73 maize and 37 whole grain rice samples (Table 2). Sixteen of the rice samples were examined as whole grain rice (paddy) and all 37 as dehusked rice (brown rice). The storage length of the maize samples was 1–3 yr, while that of the whole grain rice was from 1 to 14 yr. The geographical locations of these collected samples are presented in Fig. 1.

### 2.2. Extraction and purification

Estimation of aflatoxin in the grain samples was performed on 25 g aliquots of grain by solvent extraction followed by high-performance liquid chromatography (HPLC) with fluorescence detection. Extraction and purification protocols used in this study were as described by Wen (1996) with minor modifications. Each 25 g sub-sample was ground to a fine powder, and chloroform (125 mL) was added with constant oscillation at 100 r.p.m. for 30 min for the initial aflatoxin extraction. The extract was harvested by filtering out the insoluble debris with Whatman no. 4 filter paper. The organic extract was further purified using a chromatography column primed with florisil. Chloroform–hexane (1:1) and chloroform–methanol (9:1) solutions were added sequentially to eliminate impurities from the maize, and chloroform–methanol (9:1) was

Table 2
Aflatoxins in maize, whole grain rice and brown rice in Liaoning Province

| Grain | Samples collected | Samples tested | Samples containing aflatoxins | Mean value ($\mu g\,kg^{-1}$)[a] | Samples containing no aflatoxins |
|---|---|---|---|---|---|
| Maize | 73 | 73 | 71 | 0.99b | 2 |
| Whole grain rice | 37 | 16 | 16 | 3.87a | 0 |
| Brown rice (dehusked) | — | 37 | 36 | 0.88b | 1 |
| Total | 110 | 110 | 107 | — | 3 |

[a]The values represent total amount of aflatoxins ($B_1$, $B_2$, $G_1$, and $G_2$). The values not followed by the same letter are significantly different ($P = 0.05$) as determined by pairwise $t$-tests.

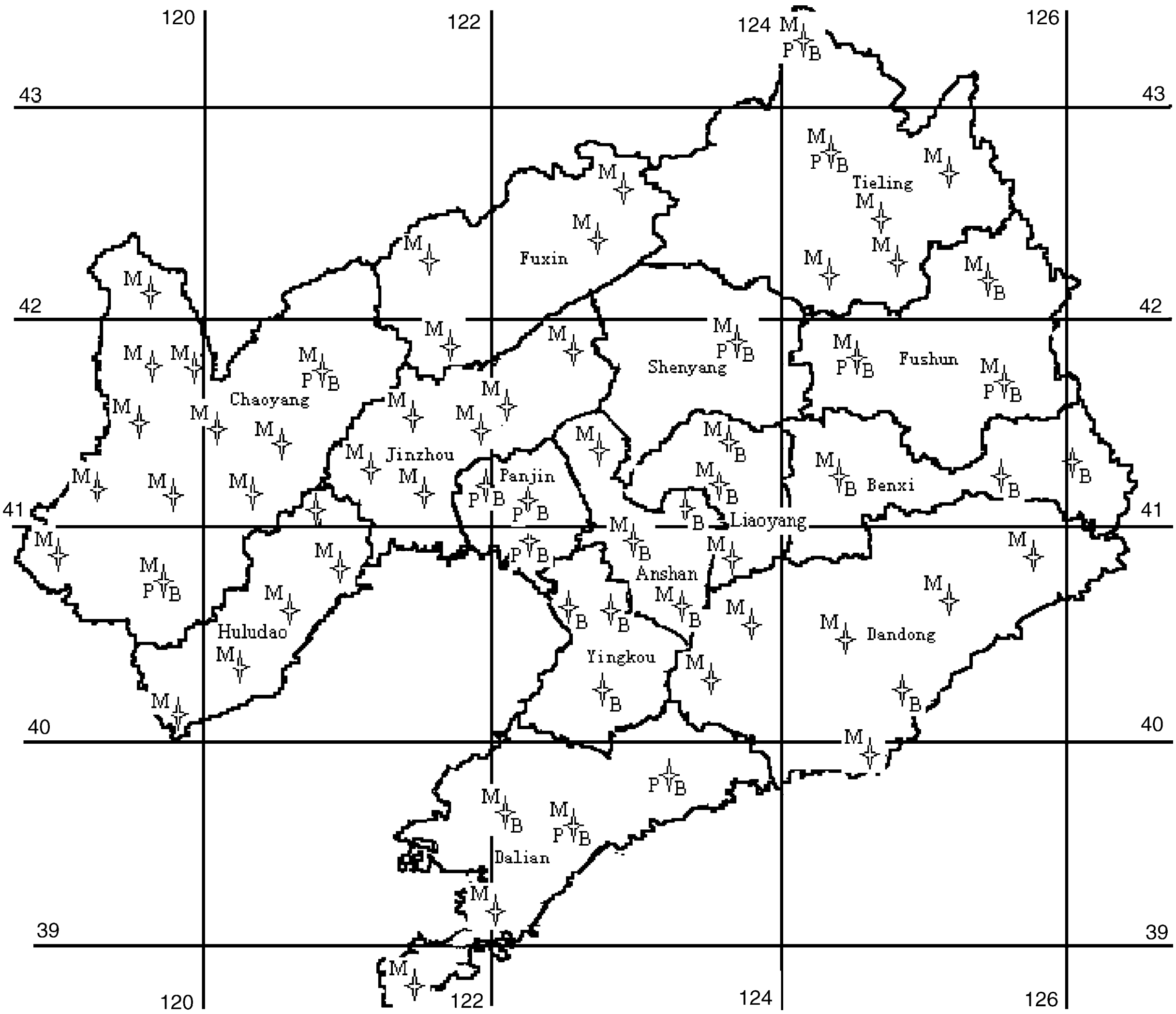


Fig. 1. Geographical locations of grain samples collected in Liaoning Province. Grid indicates the North latitude and East longitude. ✧: sampling site, M—maize, P—paddy rice, B—brown rice.

used for the whole grain rice and dehusked rice (brown rice). The purified extracts were re-dissolved in an acetone-distilled water (99:1) solution and air-dried under a hood. Finally, the extracted toxin sample was dissolved in 200 μL trifluoroacetic acid for derivatisation, and stored in a glass vial for further HPLC analysis.

### 2.3. Detection

The standards of the four aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ were a mixture of dry powder purchased from the American Romer Laboratory. The dry powder was dissolved in 5 mL acetonitrile and

Table 3
Recovery of aflatoxins $B_1$, $B_2$, $G_1$ and $G_2$ from spiked samples of maize

| Aflatoxin | Equivalent concentration added ($\mu g\,kg^{-1}$) | Mean recovery (% + SD) | Equivalent concentration added ($\mu g\,kg^{-1}$) | Mean Recovery (% + SD) |
|---|---|---|---|---|
| $B_1$ | 10 | $93.0 \pm 0.11$ | 20 | $92.7 \pm 0.16$ |
| $B_2$ | 3 | $81.2 \pm 0.16$ | 6 | $80.7 \pm 0.18$ |
| $G_1$ | 10 | $87.8 \pm 0.12$ | 20 | $86.8 \pm 0.14$ |
| $G_2$ | 3 | $90.8 \pm 0.08$ | 6 | $85.2 \pm 0.12$ |

the diluted standards contained 5 μg $B_1$, 1.5 μg $B_2$, 5 μg $G_1$ and 1.5 μg $G_2$ per mL, according to the manufacturer's manual.

HPLC with fluorescence detection was used in determining the amount of aflatoxins in the samples. HPLC analysis was performed in the Modern Analysis Test Center of the Dalian Institute of Chemical Physics, Chinese Academy of Sciences, which is one of the authoritative testing organizations in China that are fully certified by China National Accreditation Board for Laboratories (CNAL). The HPLC system is a Waters 2690 Alliance HPLC equipped with Waters 474 fluorescence detection apparatus. Chromatographic separations were performed using a stainless steel C18 reversed-phase column (250 mm × 4.6 mm i.d., 10 μm particle size). Methanol–acetonitrile–water (20:20:60) was used as the mobile phase at a flow rate of $1\,mL\,min^{-1}$. Excitation and emission wavelengths were 360 and 450 nm, respectively. Standard curves for aflatoxins $B_1$, $B_2$, $G_1$, and $G_2$ were constructed using known $B_1$, $B_2$, $G_1$, and $G_2$ standard samples in a series of concentrations. Peak areas of the excitation curves were used for quantification. The detection sensitivities of the analytical method were 0.05, 0.0074, 0.1 and $0.0074\,\mu g\,kg^{-1}$ for $B_1$, $B_2$, $G_1$, and $G_2$, respectively.

Recovery efficiency of aflatoxin extraction by the above protocol was evaluated by adding aflatoxin standards at different concentrations to uncontaminated maize samples followed by extraction, florisil column purification and HPLC analysis. The average recovery rate (over ten-replicates) for the four aflatoxins was from 80.7% to 93.0% with very low standard deviation (Table 3). The verification process demonstrated that the extraction method is reliable and accurate.

## 3. Results

### 3.1. Aflatoxin contamination in grains

Among the 110 samples collected and tested, 107 (71 maize and 36 dehusked brown rice) contained aflatoxins (Table 2). All 16 whole grain rice samples tested were found to contain aflatoxins (mean = $3.87\,\mu g\,kg^{-1}$), while with the maize, the aflatoxins content was much lower (mean = $0.99\,\mu g\,kg^{-1}$). To determine whether the aflatoxins in the whole grain rice penetrated into the rice kernels, sub-samples of all 37 whole grain rice samples were dehusked and the amount of aflatoxins determined. The dehusking was found to have removed most of the aflatoxins from whole grain rice samples although some aflatoxins had diffused through the hulls into the rice kernels. The aflatoxin content was reduced from 3.87 μg kg$^{-1}$ in whole grain rice to 0.88 μg kg$^{-1}$ in brown rice. Thus, it is a good practice to store the harvested grains for human consumption in the form of whole grain rice to reduce aflatoxin contamination. In general, the levels of aflatoxins in maize and rice were considered to be very low and far below the maximum amount allowed for food (Table 1). These results demonstrate that such grains produced in Liaoning Province are safe for human consumption and for trading.

### 3.2. Aflatoxin content and length of storage

The data in Table 4 indicate that no significant linear relationship exists in whole grain rice and brown rice between the amount of aflatoxins and the length of storage. The amount of aflatoxins in whole grain rice samples from 1 to over 10 yr ranged from 2.79 to 2.93 μg kg$^{-1}$ and peaked in the samples that were stored for 7–8 yr (6.23 μg kg$^{-1}$, Fig. 2, Table 4). With increasing storage length, the aflatoxin content in brown rice was consistently low ranging from 0.74 to 1.19 μg kg$^{-1}$ (Fig. 2, Table 4). However, in maize samples, the amount of aflatoxins significantly increased with storage length. The average amount of aflatoxins in 1-yr maize was only 0.84 μg kg$^{-1}$, while in 2-yr maize it was as high as 1.17 μg kg$^{-1}$ (Fig. 3, Table 4). Practically, no maize grains are kept in storage for more than 3 yr.

### 3.3. Occurrence of the four major types of aflatoxins in grains

All four major types of aflatoxins ($B_1$, $B_2$, $G_1$, and $G_2$) were detected in the grain samples (Table 5). The types of aflatoxins in the grains, however, were quite different. Only two maize samples contained all four types of aflatoxins ($B_1$, $B_2$, $G_1$, and $G_2$) (Table 5), which is consistent

Table 4
Aflatoxin content of maize, whole grain rice and brown rice stored for 1 yr to > 10 yr

| Grain | Years of storage | Samples tested | Samples containing aflatoxins | Mean value (μg kg$^{-1}$) | Samples containing no aflatoxins |
|---|---|---|---|---|---|
| Maize[a] | 1 | 61 | 60 | 0.84 | 1 |
| | 2 | 10 | 9 | 1.17 | 1 |
| Whole grain rice | 1–2 | 4 | 4 | 2.79 | 0 |
| | 5–6 | 4 | 4 | 3.98 | 0 |
| | 7–8 | 4 | 4 | 6.23 | 0 |
| | > 10 | 4 | 4 | 2.93 | 0 |
| Brown rice | 1–2 | 21 | 20 | 0.89 | 1 |
| | 5–6 | 7 | 7 | 0.74 | 0 |
| | 7–8 | 5 | 5 | 0.76 | 0 |
| | > 10 | 4 | 4 | 1.19 | 0 |

[a]Two maize samples of 1.5 yr and 3 yr were not included in the statistics because only one sample was collected in the year.

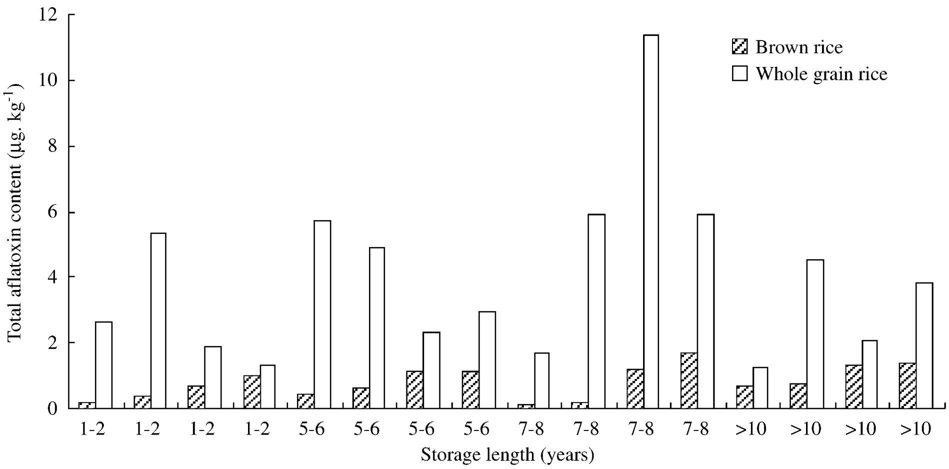


Fig. 2. Comparison of aflatoxin content in whole grain rice and brown rice in storage for one to $>10$ yr. Each pair of histograms represents data for individual samples.

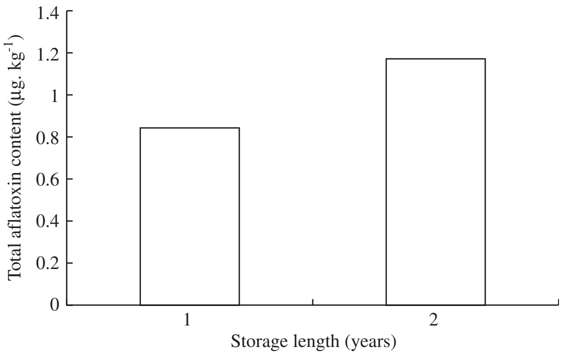


Fig. 3. Comparison of aflatoxin content in maize in storage for 1–2 yr.

with the toxin-producing profile when contaminated by *A. parasiticus* (Egel et al., 1994). There were four maize samples tested that contained only $B_1$ and $B_2$, which is consistent with the contamination profile from *A. flavus* (Cotty and Cardwell, 1999). It was interesting to find that 18 maize samples tested contained three of the four types of aflatoxins, lacking either $G_2$, $B_2$, or $B_1$. In many maize samples only two or even a single type of aflatoxin ($G_1$, $B_1$, or $B_2$) were detected (Table 5). Only aflatoxin $G_1$ was detected in the majority of the rice grain samples, which accounted for 93.7% of the 16 whole grain rice tests and 91.7% of the 36 dehusked brown rice tests (Table 5). Only aflatoxins $B_1$ and $G_1$ were detected in three of the 36 brown rice tests, and only aflatoxin $G_1$ and $G_2$ were detected in one of the 16 whole grain rice tests. Aflatoxin $B_1$ and/or $B_2$, however, were not detected in whole grain rice samples.

Significant differences were observed in the amount and type of aflatoxins in maize samples stored at two locations (KD, in Dandong—3 sampling sites representing length of storage for 1, 2, and 3 yr and FX, in Fuxin—2 sampling sites representing length of storage for 1 and 2 yr, Figs. 1 and 4). With increasing storage time, the levels of aflatoxin $B_1$ were higher. Aflatoxin $G_1$ was

Table 5
Occurrence of the four major type of aflatoxins in maize, whole grain rice and brown rice in Liaoning Province

| | Maize | | Whole grain rice | | Brown rice | |
|---|---|---|---|---|---|---|
| | No. of samples | (%) | No. of samples | (%) | No. of samples | (%) |
| $B_1$, $B_2$, $G_1$, $G_2$ | 2 | 2.8 | 0 | 0.0 | 0 | 0.0 |
| $B_1$, $B_2$, $G_1$ | 15 | 21.1 | 0 | 0.0 | 0 | 0.0 |
| $B_1$, $G_1$, $G_2$ | 2 | 2.8 | 0 | 0.0 | 0 | 0.0 |
| $B_2$, $G_1$, $G_2$ | 1 | 1.4 | 0 | 0.0 | 0 | 0.0 |
| $B_1$, $B_2$ | 4 | 5.7 | 0 | 0.0 | 0 | 0.0 |
| $B_1$, $G_1$ | 14 | 19.7 | 0 | 0.0 | 3 | 8.3 |
| $B_2$, $G_1$ | 19 | 26.8 | 0 | 0.0 | 0 | 0.0 |
| $G_1$, $G_2$ | 1 | 1.4 | 1 | 6.3 | 0 | 0.0 |
| $B_1$ | 7 | 9.9 | 0 | 0.0 | 0 | 0.0 |
| $B_2$ | 1 | 1.4 | 0 | 0.0 | 0 | 0.0 |
| $G_1$ | 5 | 7.0 | 15 | 93.7 | 33 | 91.7 |
| Total | 71 | 100.0 | 16 | 100.0 | 36 | 100.0 |

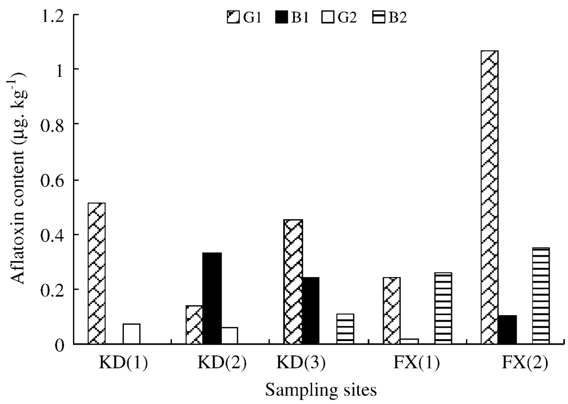


Fig. 4. Four major types of aflatoxins in maize samples collected from storages with grain held for 1, 2 and 3 yr (location KD), and 1 and 2 yr (location FX). See Fig. 1 for locations of KD (Dandong) and FX (Fuxin). Note that the numbers in parentheses indicates the length of storage in years.

dominant in most of the samples, and $G_1$ and $B_2$ were dominant in the samples collected at both sites at location FX covering 1 and 2 yr storage.

## 4. Discussion

Aflatoxin contamination and *A. flavus* infection are often associated with drought and temperature (Ju, 1980; Moreno and Kang, 1999). Liaoning Province is located between 38°N and

Table 6
Temperature, relative humidity and rainfall in Liaoning Province

| | Yearly average temperature (°C) | Yearly high temperature (°C) | Yearly relative humidity (%) | Yearly precipitation (mm) |
|---|---|---|---|---|
| Province-wide | 4.8–10.4 | 29.2 | 65 | 485–1130 |
| Dalian (39°N/122°E)[a] | 10.2 | 27.0 | 67 | 550–950 |
| Tieling (43°N/124°E) | 7.8 | 28.6 | 63 | 700 |
| Benxi (41°N/126°E) | 6.1–7.8 | 27.1 | 64 | 800–900 |
| Chaoyang (41°N/120°E) | 5.4–8.7 | 30.1 | 52 | 450–580 |

[a]The four cities represent locations in the four directions (South, North, East and West) in Liaoning Province. Numbers in parentheses indicate the latitude and longitude of each geological location.

44°N latitude (38°43′–43°26′) and between 118°E and 126°E longitude (118°53′–125°46′). It covers a large area with diverse weather conditions. The temperature is relatively cool all year around (Table 6). The temperature is higher in the southern region and lower in the northern region. Dry and hot weather occurs in the western region (Chaoyang) and more precipitation was recorded in the southern and eastern regions (Table 6). The extremely low level of aflatoxin contamination in maize and rice grains might be due, in particular, to the fact that the cooler weather conditions in northern China discouraged the growth of these aflatoxigenic fungi. None of the samples tested had higher aflatoxin content than the regulated maximum amount allowed. Though aflatoxin content in these samples was below the governmental regulated level, an incidence of aflatoxicosis was reported once (Dai, 1997). Since maize and rice are the main diet of the local population and are consumed in large quantities, aflatoxin contamination could be a serious problem even at low levels (Paranagama et al., 2003). Improvement in storage conditions to prevent grain spoilage and reduce aflatoxin contamination is recommended.

The aflatoxin content in the samples stored for over 10 yr was less than those stored for 7–8 yr. The reason for the decline over a longer storage period is unclear. Some researchers (Paranagama et al., 2003) found that the aflatoxigenic fungi and aflatoxin formation might be inhibited by some synthetic pesticides or natural protectants during storage. Not only did some microbial populations decline in number, but the amount of aflatoxins was reduced (Ju, 1980). This might help to partially explain our observation in this study, though we did not have detailed information on the pesticide application to the grain that was sampled. Aflatoxins, however, showed an increasing trend with the length of storage, with higher levels detected in the samples stored for 7–8 yr than those stored for 1–6 yr.

The level of aflatoxins in maize was lower than that in whole grain rice. The low aflatoxin content in maize might be due to better storage conditions than for whole grain rice. Maize was kept in modern storehouses with sufficient ventilation to reduce moisture, while most whole grain rice was stored in old-fashioned storehouses with poor ventilation. The low aflatoxins in brown rice might be related to the unique physical structure of the rice hull that plays an important role in protecting seed kernels from being infected by the aflatoxigenic moulds (Fig. 2). This also demonstrated the historical wisdom of farmers in storing whole grain rice and consuming polished rice rather than brown rice.

In general, the toxigenic *A. flavus* strains produce aflatoxins $B_1$ and $B_2$ (Cotty and Cardwell, 1999), while *A. parasiticus* strains produce $B_1$, $B_2$, $G_1$, and $G_2$ (Egel et al., 1994). Aflatoxins $B_1$ and

$B_2$ are the most potent and the most common toxins in contaminated grains in most countries and are the focus of research (Wen, 1996; Cotty, 1997; Li, 1997). In contrast to the general aflatoxin-producing profile, data presented in Table 5 showed that the types of aflatoxins detected in the samples were quite uncommon. Only two maize samples were found to contain four types of aflatoxins ($B_1$, $B_2$, $G_1$ and $G_2$) and four samples to contain only B group toxins ($B_1$ and $B_2$). The rest of the samples contained toxins which were inconsistent with either the *A. flavus* or *A. parasiticus* aflatoxin-producing profiles. Biochemically, it is unlikely that G group toxins can be produced without B group toxins, based on our current understanding of the aflatoxin biosynthetic pathway of the known aflatoxigenic fungi (Yu et al., 2004b). In order to verify the accuracy of the data, the sample extraction and HPLC procedures were repeated and the types of toxins, as well as their content, were double-checked. It is possible that the fungi grown on rice might be a new species other than the two aflatoxigenic species (*A. flavus* and *A. parasiticus*). Preliminary data suggested that both *A. flavus* and *A. parasiticus* colonize maize samples. Other aflatoxigenic fungi may exist in rice samples and need to be identified or the rice plant might have altered the aflatoxin-producing pattern by plant–fungus interaction. Previous reports (Greene-Mcdowelle et al., 1999; Wright et al., 2000) indicated that certain volatile compounds generated by plants inhibited aflatoxin formation. The plant lipoxygenase in maize and peanuts and its product, the 13(S)-hydroperoxide derivative, were also shown to interfere with aflatoxin formation (Burow et al., 2000; Wilson et al., 2001). The rice grain might contain or have produced certain enzymes or chemicals that inhibited the production of aflatoxin or a type of aflatoxin (such as $B_1$). Such further studies are beyond the scope of this report.

Investigation on aflatoxin contamination in Liaoning Province demonstrated that the average aflatoxin content ($0.99\,\mu g\,kg^{-1}$ in maize and $0.88\,\mu g\,kg^{-1}$ in brown rice) was much lower than other regions in Asia (Tang et al., 1998; Tang, 1999) and the regulated safe consumption level established by most countries. Large numbers of samples were collected over the entire Province, and we are confident in concluding that the grains produced in Liaoning Province are safe for consumption and for national and international trading without restriction.

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## References

Bennett, J., Goldblatt, L.A., 1973. The isolation of mutants of *Aspergillus flavus* and *A. parasiticus* with altered aflatoxin producing ability. Sabouraudia 11, 235–241.

Breckenridge, C., Samarajeeua, U., Arsecularatne, S.N., 1986. Aflatoxin contamination and moisture levels in Sri Lankan market rice. Journal of National Science Council, Sri Lanka 14, 173–180.

Burow, G.B., Gardner, H.W., Keller, N.P., 2000. A peanut seed lipoxygenase responsive to *Aspergillus* colonization. Plant Molecular Biology 42, 689–701.

Cleveland, T.E., Bhatnagar, D., 1992. Molecular strategies for reducing aflatoxin levels in crops before harvest. In: Bhatnagar, D., Cleveland, T.E. (Eds.), Molecular Approaches to Improving Food Quality and Safety. Van Nostrand Reinhold, New York, pp. 205–228.

Cotty, P.J., 1997. Aflatoxin-producing potential of communities of *Aspergillus* section Flavi from cotton producing areas in the United States. Mycological Research 101, 698–704.

Cotty, P.J., Cardwell, K.F., 1999. Divergence of West African and North American communities of *Aspergillus* section Flavi. Applied and Environmental Microbiology 65, 2264–2266.

Dai, J., 1997. Aflatoxin toxicosis diagnosis. Chinese Animal Infection 93, 47–48.

Dowling, F.S., 1997. Fumonisin and its toxic effects. Cereal Foods World 42, 13–15.

Egel, D.S., Cotty, P.J., Elias, K.S., 1994. Relationships among isolates of *Aspergillus* section Flavi that vary in aflatoxin production. Phytopathology 84, 906–912.

Ellis, W.O., Smith, J.P., Simpson, B.K., 1991. Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection and methods of control. Critical Reviews in Food Science and Nutrition 30, 403–439.

Greene-Mcdowelle, D.M., Ingber, B., Wright, M.S., Zeringue Jr., H.J., Bhatnagar, D., Cleveland, T.E., 1999. The effects of selected cotton-leaf volatiles on growth, development and aflatoxin production of *Aspergillus parasiticus*. Toxicon 37, 883–893.

Hansen, T.J., 1993. Quantitative testing for mycotoxins. American Association of Cereal Chemists 38, 346–348.

Ilago, L.L., Juliano, B.O., 1982. Colonization and aflatoxin formation by *Aspergillus* spp. on whole grain rice differing in endosperm properties. Journal of Science of Food and Agriculture 33, 97–102.

Ju, N., 1980. Aflatoxin. Light Industry Press, Beijing, China.

Li, Y., 1997. Determination and control of aflatoxin in feedstuff. Grain and Feedstuff Industry 8, 39–40.

Liu, X., Yin, X., Li, Y., Meng, Z., 1981. Investigation on toxic-producing of *A. flavus* in grains in different region, China. Journal of Chinese Medicine Academic 3, 266–269.

Moreno, O.J., Kang, M.S., 1999. Aflatoxins in maize: the problem and genetic solutions. Plant Breeding 118, 1–16.

Nesci, A., Rodriguez, M., Etcheverry, M., 2003. Control of *Aspergillus* growth and aflatoxin production using antioxidants at different conditions of water activity and pH. Journal of Applied Microbiology 95, 279–287.

Paranagama, P.A., Abeysekera, K.H.T., Abeywickrama, K., Nugaliyadde, L., 2003. Fungicidal and anti-aflatoxigenic effects of the essential oil of *Cymbopogon citrata* (DC.) Stapf. (lemongrass) against *Aspergillus flavus* Link. isolated from stored rice. Letters in Applied Microbiology 37, 86–90.

Paster, N., 1995. Fungi in stored grain and animal feeds: their occurrence and harm caused to animals. Israel Journal of Veterinary Medicine 50, 49–53.

Pitt, J.I., 2000. Toxigenic fungi and mycotoxins. British Medical Bulletin 56, 184–192.

Resnik, S., Neira, S., Pacin, A., Martinez, E., Apro, N., Latreite, S., 1996. A survey of the natural occurrence of aflatoxins and zearalenone in Argentina field maize 1983–1994. Food Additives and Contaminants 13, 115–120.

Tang, J., 1999. Research progress of aflatoxin in Guangxi. Journal of Chinese Veterinary Medicine 5, 33–35.

Tang, Z., Li, G., Kang, Y., Zhong, Z., 1998. The investigation of the groundnut contaminated by *Aspergillus flavus* before harvest. Fujian Journal of Agriculture Science 13, 25–28.

Wen, J., 1996. Quantitative analysis of HPLC and separation and purification of siliconmagnesium absorbent column for aflatoxin $B_1$ of corn. Food Science 17, 68–70.

Wilson, R.A., Gardner, H.W., Keller, N.P., 2001. Cultivar-dependent expression of a maize lipoxygenase responsive to seed infesting fungi. Molecular Plant–Microbe Interactions 14, 980–987.

Wright, M.S., Greene-Mcdowelle, D.M., Zeringue Jr., H.J., Bhatnagar, D., Cleveland, T.E., 2000. Effects of volatile aldehydes from *Aspergillus*-resistant varieties of corn on *Aspergillus parasiticus* growth and aflatoxin biosynthesis. Toxicon 38, 1215–1223.

Xiao, D., 1988. Contamination research of aflatoxin in Chinese peanut. Chinese Oil Plants 2, 85–87.

Yu, J., Bhatnagar, D., Cleveland, T.E., 2004a. Genetics and biochemistry of aflatoxin formation and genomics approach for eliminating aflatoxin contamination. In: Romeo, J.T. (Ed.), Recent Advances in Phytochemistry: Secondary Metabolism in Model Systems, vol. 38. Elsevier, Amsterdam, pp. 224–242.

Yu, J., Chang, P.-K., Ehrlich, K.C., Cary, J.W., Bhatnagar, D., Cleveland, T.E., Payne, G.A., Linz, J.E., Woloshuk, C.P., Bennett, J.W., 2004b. Clustered pathway genes in aflatoxin biosynthesis. Applied and Environmental Microbiology 70, 1253–1262.

Zhang, P., Zhang, J., Han, G., 2003. Rapid detection of aflatoxin contamination in feedstuff. Modern Agriculture 5, 40.